IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Peter Richardson	Art Unit	: 1623
Serial No.	: 10/537,564	Examiner	: Lawrence E. Crane, Ph.D.
Filed	: August 28, 2006	Conf. No.	: 4551
Title	: USE OF SPONGOSINE FOR THE TREATMENT OF PAIN		

Commissioner for Patents
P.O. Box 1450
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DECLARATION OF PETER RICHARDSON UNDER 37 C.F.R. § 1.132

Peter Richardson declares as follows:

1. I have BA in Biochemistry from the University of Oxford (1976), a MA from the University of Oxford (1979), and a Ph.D. in Biochemistry from the University of Cambridge (1979). My *curriculum vitae* is attached as Exhibit A.

2. I am an inventor of the above-captioned patent application.

Spogosine and Rat Model of Inflammatory Pain

3. Rats injected in the hindpaw with a mixture of *Mycobacterium butirricum* emulsified in light mineral oil (complete Freund's adjuvant, CFA) develop a severe polyarthritis which shares some features with human rheumatoid arthritis (RA), such as swelling of the extremities, cartilage degradation, loss of joint function and lymphocyte infiltration into diseased joints. The ability of spongosine to reverse allodynia (painful response to a normally innocuous stimuli) resulting from immunization with CFA (as a model of inflammatory pain), was assessed using osmotic minipumps to administer spongosine.

4. The spongosine was delivered using one or two minipumps per animal, each delivering 0.2mg/kg/day. Thus, spongosine was administered at a rate of 0.2 mg/kg/day (one

pump; 1P) or 0.4 mg/kg/day (two pumps; 2P). In parallel experiments it was shown that these administration protocols resulted in steady state concentrations of 7 nM and 13 nM sponogosine, respectively in the two groups (n = 8 or 9) of animals.

5. The effect of spongosine on the development of mechanical allodynia resulting from immunization with CFA was measured using the Dynamic Plantar Aesthesiometer (DPA, Ugo Basile, Varese, Italy), which is an automated version of the von Frey hair assessment. Allodynia was detected as a reduction in the paw withdrawal threshold (i.e., the animal becomes more sensitive to the pressure induced pain as a result of the inflammation). As shown in Figure 1, below, the reduction in withdrawal threshold observed with untreated animal (vehicle treated) was greatly reduced in animals treated with spongosine at 0.2 mg/kg/day (1P) or 0.4 mg/kg/day (2P). Thus, both doses of spongosine essentially eliminated the development of mechanical allodynia in a chronic rat model of adjuvant arthritis. The data are means ± standard error mean (SEM) of 8 or 9 animals. Statistics are below the figure.

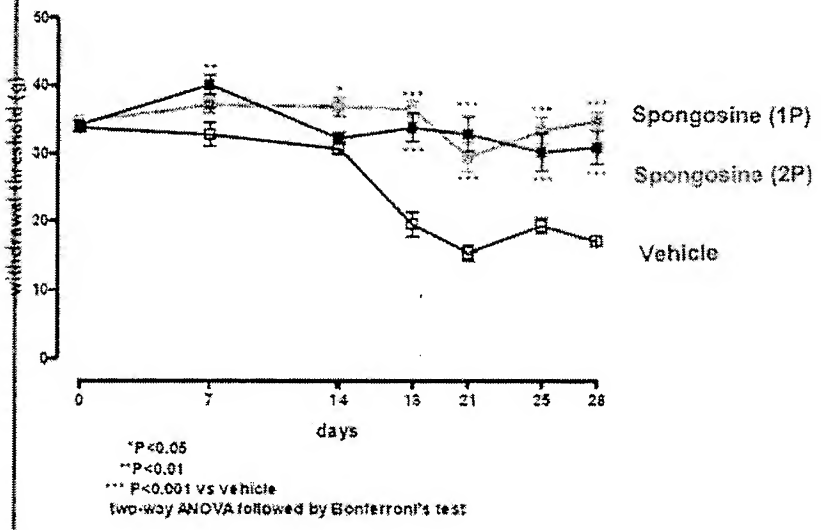

FIGURE 1

6. As explained in the specification of the above-captioned application at paragraph [0009], it has been reported that the Kd of spongonsine as the A1 receptor is 340 nM and that the

Kd of spongosine at the A2A receptor is 1,400 nM (Daly et al. 1993 *Pharmacology* 46:91). It has also been reported that agonists of the A1 receptor act as analgesics (Sawynok 1998 *European Journal of Pharmacology* 347:1) (see specification of the above captioned application at paragraph [0005].

7. The fact that spongosine was effective in inhibiting pain perception in a rat model of inflammatory pain at steady state concentrations, 7 nM and 13 nM, that are far below the Kd of spongosine for the A1 and A2A receptors is surprising. Without being bound by any particular theory, it appears that in certain tissues, such as epithelia, tissue damaged by physical, chemical or biological trauma, and those tissues undergoing an inflammatory response, the pH is lower than that of other tissues. The lower pH alters the binding affinity of spongosine for adenosine receptors such that spongosine is selective for the A2A adenosine receptor in such tissues. This allows the unexpected alleviation of pain and inflammation by spongosine at a plasma concentration that is too low to activate A1 and A2A adenosine receptors in other tissues thereby avoiding such negative side-effects such as bradycardia and hypotension.

Spogosine and Rat Model of Neuropathic Pain

8. In rats ligation of the sciatic nerve results in a reduction in pain threshold analogous to that seen in neuropathic pain in humans (Bennett et al. 1988 *Pain* 33:87). The ability of spongosine to reverse allodynia associated with sciatic nerve ligation was assessed in rats using osmotic minipumps to administer spongosine.

9. The spongosine was delivered using one or two minipumps per animal, each delivering 0.2mg/kg/day, and so spongosine was administered at a rate of 0.2 mg/kg/day (one pump; 1P) or 0.4 mg/kg/day (two pumps; 2P). In parallel experiments it was shown that this administration protocol resulted in steady state concentrations of 7 nM and 13 nM sponogosine respectively in the two groups (n=8/9) of animals.

10. After ligation of the nerve in rats, allodynia was measured as described above in paragraph 5. As shown in Figure 2, below, the reduction in paw withdrawal threshold was

reduced in a dose dependent fashion by the administration of spongosine (statistical analysis below figure). As shown in Figure 3, below, a similar effect was not observed in the contralateral paw, i.e., the paw in which the nerve was not ligated (data are mean ± SEM for 8 or 9 animals). This demonstrates that spongosine was not affecting the mechanical function of paw withdrawal.

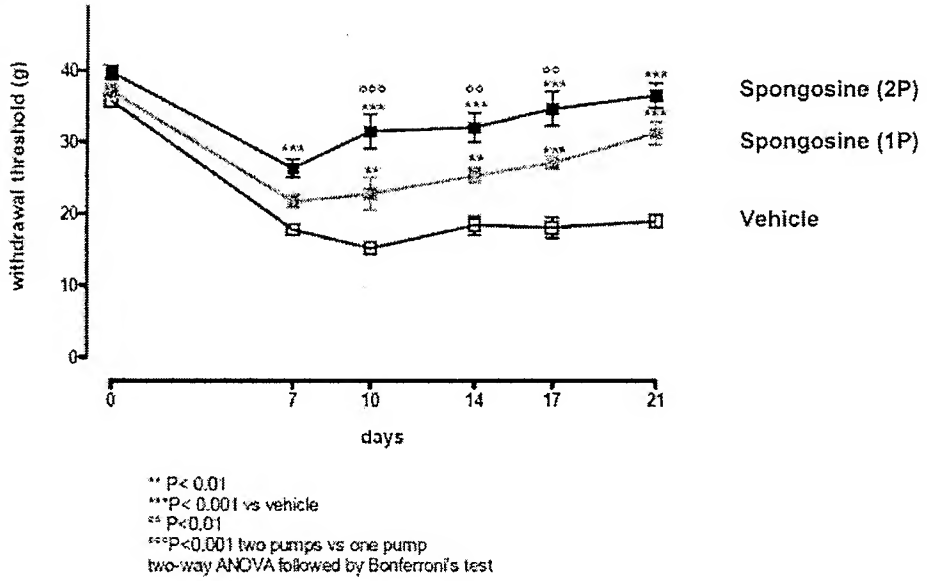

FIGURE 2

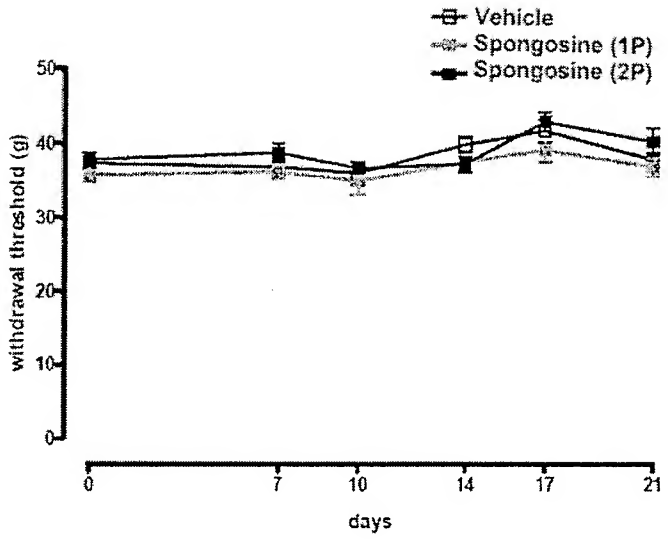

FIGURE 3

11. The fact that spongosine was effective in inhibiting pain perception in a rat model of neuropathic pain at steady state concentrations (7 nM and 13 nM) that are far below the Kd of spongosine for the A1 and A2A receptors is surprising.

Clinical Trial of Spongosine for Treatment of Diabetic Neuropathy

12. In a Phase 2A clinical trial, hypertensive patients with painful diabetic neuropathy were administered 7 mg of spongosine three times per day for 28 days. In this trial the average weight of the indicated subjects was 97 kg, and the weight of the subjects ranged from 70-133 kg. Thus, the average dose was 0.07 mg/kg administered three times per day with a range of 0.1 to 0.05 mg/kg administered three times per day. This treatment regime resulted in peak plasma concentrations of spongosine between 150 nM and 360 nM. In a separate study using cloned human receptors the Ki of spongosine for the A1 receptor was found to be 10,000 nM and the Kd for the A2A receptor was found to be 1,400. Briefly, for the human A1 receptor, the inhibition of forskolin stimulated cAMP accumulation in HEK293 cells transfected with the human A1 receptor was assessed, and 50% of maximal inhibition of cAMP (defined using the typical A1

agonist CCPA) accumulation was achieved at approximately 10,000 nM. For the human A2A receptor the Kd was determined by displacement of ^{3}H-labelled CGS21680 binding to HEK293 cells stably transfected with the human A2A receptor.

13. The subjective pain score (measured on the Likert scale where 0 is no pain and 10 is unimaginable pain) was assessed for treated and untreated patients using a daily diary and the average weekly score was plotted. The results of this study are presented in Figure 4, below, in which data are expressed as means ± 95% confidence intervals. Detailed statistical analysis is presented in Table 1, below. As shown in Figure 4, treatment with spongosine resulted in a statistically significant reduction in pain compared to subjects treated with a placebo. This reduction in pain was observed at a peak plasma concentration well below the Ki of spongosine for the human A1 receptor and well below the Kd of spongosine for the human A2A receptor.

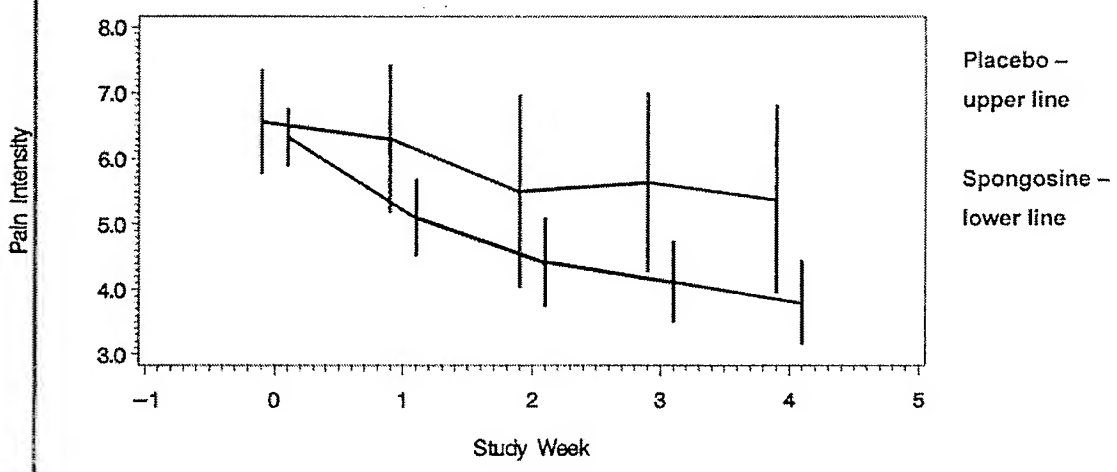

FIGURE 4

Table 1

	Baseline		Change at Week 4	
	Spongosine	Placebo	Spongosine	Placebo
N	37	15	37	15
Mean (Std Dev)	6.33 (1.32)	6.56 (1.43)	-2.53 (1.57)	-1.18 (1.57)
Median	6.4	6.5	-2.6	-0.6
Range	4.2 – 9.3	4.0 – 9.8	-6.5 – 0.0	-4.7 – 0.8
Estimate (Conf. Int.)	-1.19 (-2.11, -0.2)			
p-value	0.0124*			

Difference from placebo (derived from ANCOVA model with baseline, country and treatment) greater than primary endpoint
* Indicates statistical significance at the 5% level (two-sided)

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: _____

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Peter Richardson, Ph.D.